IMMUNITY IN BRUCELLOSIS[1]

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The principal purpose of this review is to bring together many of the data that are related directly or indirectly to the state of active immunity to brucellosis in animals and man, to critically analyze the data as to their significance, and to point out if possible the nature of the immunity. The term "active immunity" will be interpreted in this review with the same meaning so well expressed in the following definition given by Rivers (1941): "Active immunity is a state of resistance to infection engendered by a normal spontaneous attack of an infectious disease, by the experimental or intentional production of the disease or a modified form of it, or by the injection of vaccines." Data having a bearing on two possible methods by which an actively acquired immunity may be engendered will be reviewed. One is associated with recovery from natural or experimental infection and the other pertains to the injection of living or dead organisms, or their antigenic constituents. Since the only sure indicator of active immunity to brucellosis is resistance to infection, the data and observations bearing on the subject will be discussed with this objective in view.

THE BOVINE

Active immunity acquired through infection

Approximately 55 years have elapsed since bovine brucellosis was recognized as a disease entity and its study undertaken in a scientific manner. In view of the fact that the chief symptom of the disease in the cow is the premature expulsion of the fetus, Bang (1906) and many of those who followed devoted most of their efforts toward the symptom and its prevention rather than toward the diseased animal. Even diagnostic procedures were first studied with the object in view of determining whether an animal would abort or the abortion was due to *Brucella abortus*. Preventative measures and likewise active immunity were considered and studied from the standpoint of preventing the occurrence of the symptom. Bang, the master observer, permitted little to escape his attention and was not long in noting that many infected cows aborted only once. He speculated on the possibility of an immunity against abortion being acquired from infection.

Most of the essential data that have accumulated during the past 50 years and which serve as a basis for establishing proof that it is possible for cattle to acquire an immunity to brucellosis through infection were not collected and recorded with this object in view. Nevertheless, when the data are fitted together and considered in this direction, they furnish adequate proof that cattle very often acquire immunity to brucella infection.

The view has been expressed by many that bovine brucellosis is of the nature of a chronic disease and, since there are few, if any, examples of active immunity

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in infectious diseases of this character, one should not expect to find any active immunity to arise either after recovery or from vaccinal agents. Such asthenic reasoning would seem out of place in the twentieth century.

As early as 1916 data were collected by the writer from experiments on new-born calves which indicated that they were capable of resisting infection. The calves were exposed to infection either by nursing infected udders or by being fed live organisms added to milk. Most of the calves failed to produce demonstrable antibodies in the blood serum after exposure and, if they were present at the beginning of the feeding experiment, they disappeared within four to twelve weeks. No attempt was made at the time to recover brucella from the tissues of the calves. However, another study (1924) was conducted on 11 calves which had been exposed to infection during the nursing period, and all of which had shown serum antibodies for a short period. No evidence of persisting infection was found when the animals were slaughtered during the first pregnancy or after parturition. During the period of observation, 14 control animals housed on the same premises also remained free from infection.

In connection with the appearance of brucella antibodies in the blood serum of new-born calves, it was pointed out by Little and Orcutt (1922) that they are derived from colostrum containing such antibodies when ingested shortly after birth.

Carpenter (1924) added an important chapter to the story by demonstrating that brucella can be recovered from many organs of calves during and shortly after the feeding of milk containing the live organisms. The most interesting part of his finding was that the organisms disappeared from the tissues in the fifth week after the feeding was discontinued.

Quinlan (1923) made a study of 40 calves, 15 of which were infected dams, to determine their susceptibility to brucella infection by feeding them infected milk over a period of several weeks. Only 8 of the calves showed specific agglutinins in their blood, and then for a short time only during the period of observation; although many of them were kept under observation for as long as two years. These observations furnish additional proof of the resistance of calves to infection by brucella; and that the resistance during the exposure period does not necessarily come from the ingestion of colostrum containing specific antibodies.

Fitch and associates (1941) collected 56 female calves from brucella-infected dams to determine whether such calves, if infected, would carry the infection through to maturity and suddenly manifest evidence of the disease. The only known exposures to infection were at the time of birth and by way of infective milk during the first week of nursing. The group of animals was observed over a period of one to six parturitions. In no instance was there any evidence of infection continuing from birth or of its sudden appearance after these animals had become mature.

When one considers the results of all of the investigations together, there can be found few, if any, exceptions to the rule that calves up to a certain age possess a high degree of resistance to infection by brucella. Furthermore, the calf does not necessarily acquire its resistance toward infection as a result of ingesting

colostrum containing specific brucella antibodies. The calf from a non-infected cow, and not receiving colostrum high in brucella antibodies, appears to be just as resistant to infection up to a certain age as those that do.

There is one very important possible difference in the status of calves exposed and not exposed to infection that no one has yet thoroughly investigated. It is the difference in their susceptibility toward infection after they have reached breeding age. In conducting immunization experiments on young or mature cattle it would seem imperative, before declaring the efficacy of any immunizing agent, to know whether exposure during an early age had already left the animals with a high degree of immunity.

There came from one experiment conducted by Birch and Gilman (1925) an indication that calves, when exposed to infection at an early age, may not develop a sufficient immunity to protect them against an overwhelming experimental infection during adult life. In this particular experiment 9 pregnant heifers, 8 of which as calves had been in contact with infected animals, were exposed to infection three times a week for a period of three months by placing a suspension of *B. abortus* in the feed. Only one of the 8 resisted infection. The exception failed even to develop a high agglutination titer during the time of observation.

Although most young calves possess a high resistance to infection, it is well known that a very small percentage do become infected under natural conditions and remain so to maturity.

There may be no connection between the state which accounts for the resistance of young calves to brucella infection and that which is brought into action in adult animals as a result of exposure to infection. Two entirely different immunological phenomena may be involved. On the other hand, it is of interest to note that both resistant calves and adult animals do not develop serum antibodies unless exposed to or injected with massive doses of the organism. When antibodies do appear they remain for only a short time. This phenomenon appears to be associated with the presence of an active immunity to brucellosis in laboratory animals and human beings as well as in cattle, and will be pointed out in other parts of the review.

There is another period in the life of the bovine species when it manifests a high degree of resistance to brucella infection. This occurs during the period when the mature heifer and cow are non-pregnant. This fact was clearly demonstrated by Edington and Donham (1939) in a well-conducted series of experiments on pregnant and non-pregnant heifers and non-pregnant cows. The investigators exposed 12 pregnant and 15 non-pregnant heifers, and 6 non-pregnant cows to very large numbers of *B. abortus* organisms either by way of the eye, mouth, or vagina. In no instance was there any evidence to indicate that the organism had established itself in the non-pregnant animals. Of the 12 pregnant ones, 11 became infected and aborted. Seven of the heifers and the 6 cows exposed prior to breeding were again exposed to a large dose of *B. abortus* organisms during the subsequent period of gestation. Again, these animals failed to develop the disease. Although most of the animals developed a high agglutination

titer after exposure to the massive doses of organisms, there was a rapid decline in the titers in subsequent months.

The failure of the pregnant animals to become infected after the second exposure can be attributed only to the operation of an active immunity resulting from exposure to live organisms when they were non-pregnant. It would be rare indeed for all members of such a large group of normal pregnant animals to resist infection by any other means. The low susceptibility of non-pregnant heifers and cows may not be of the nature of an acquired immunity, but it may be related to the same state which exists in young calves, and which protects them from infection,—a state which is unfavorable for the growth and persistence of the invading organisms.

It has been observed by many that the incidence of brucella infection appears to decrease after the disease has been present in a herd for many years, provided no additions are made to the herd from the outside. This occurrence has been attributed to "decreased virulence" of the organism in the animals in the herd. It would, however, be just as logical to assume that the decrease in the incidence of new infections was due to an actively acquired immunity resulting from exposure to infection at an early age. In this connection considerable data from experimental infection studies have been obtained that have a direct bearing on the occurrence of active immunity under natural conditions. Rettger and associates (1926) exposed 26 bred and unbred heifers to brucella infection by either the oral, subcutaneous or urethral route. Their protocols show that 12 (54 per cent) failed to become infected, and a large percentage failed even to develop specific serum antibodies. It is hardly conceivable that the animals failed to become infected because the number of organisms to which they were exposed was too small. A high degree of immunity must have been present in many of the animals before exposure, or was acquired immediately as a result of the exposure.

In an experiment conducted at the University of Wisconsin with the purpose of determining the influence of an adequate and low mineral intake in cattle on their susceptibility to brucellosis by artificial exposure, Hadley and Welsh (1931) obtained data indicating that an active immunity was operating in some of the animals used in the experiment. Of a total of 33 in the two groups, 13 failed to become infected. Many of the 13 developed specific serum agglutinins in low titer for only a short time. The animals were kept under observation for two years and during this time 29 (88 per cent) had ceased to show any evidence of being infected as indicated by the absence of specific serum agglutinins and by negative bacteriological findings. Later, Beach and Humphrey (1935) used 13 of these animals in an experiment to determine their resistance to infection, the outcome of which will be discussed later.

Huddleson and Smith (1931) studied a large number of animals in a dairy herd for a period of four to eight years and obtained data indicating the existence of active immunity in many to brucella infection. During this period, the serum agglutination test was positive in a dilution of 1:100 or higher in 189 animals. A considerable number of these aborted. There were many opportunities for all

TABLE 1

Agglutination titers of 13 cows following exposure to B. abortus, the cows having been exposed to infection 5 years previously

Beach and Humphrey (1935)

COW NO.	1933					1934						
	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	April	May	June	July
4	– – – – –	– – – – –	– – – – –	– – – – –	– – – – –	– – – – –	– – – – –	– – – – –	– – – – –	– – – – – *	– – – – –	– – – – –
38	*P T* – – –	– – – – –	*P T* – – –	*P T* – – –	+ + *P P* –	+ *P T T* –	+ *P T* – –	+ *P T* – –	+ + + *P T* *	+ + + *P T*	+ + *P T* –	+ + *P T* –
44	*P T* – – –	– – – – –	– – – – –	*T* – – – –	*T T* – – –	*T T* – – –	*T* – – – –	*T* – – – – *	*T* – – – –	*T* – – – –	– – – – –	– – – – –
28	*P T* – – –	*T* – – – –	*T T* – – –	+ *P T* – –	+ *P P T* –	+ *P T* – –	*T* – – – –	*P T* – – –	*P T* – – – *	*P T* – – –	– – – – –	– – – – –
40	*P T* – – –	– – – – –	*T* – – – –	*P T* – – –	*T T* – – –	*T T* – – –	*T* – – – –	*T T* – – – *	*P T* – – –	*P T* – – –	*T* – – – –	*T T* – – –
36	*P* – – – –	– – – – –	– – – – –	– – – – –	+ + *P P* –	*P T* – – –	*T* – – – –	– – – – – *	– – – – –	– – – – –	– – – – –	– – – – –
12	*P T* – – –	*T* – – – –	– – – – –	*T* – – – –	+ *P T* – –	*P P T* – –	*T* – – – –	*P T* – – – *	+ *P* – – –	*T* – – – –	– – – – –	*P T* – – –
8	– – – – –	– – – – –	– – – – –	*T* – – – –	*T T* – – –	*P T T* – –	– – – – –	*T* – – – –	– – – – –	– – – – –	*P* – – – –	*T T* – – –
17	*T* – – – –	*T* – – – –	*P T* – – –	+ *P T* – –	+ *P T T* –	*T T* – – –	– – – – –	– – – – –	– – – – –	– – – – – *	– – – – –	– – – – –
5	*P T* – – –	*T* – – – –	+ + + + +	+ + + + + †								
31	– – – – –	– – – – –	– – – – –	– – – – –	– – – – –	– – – – –	– – – – –	– – – – – *	– – – – –	– – – – –	– – – – –	– – – – –
33	*P T* – – –	*T* – – – –	*P T* – – –	*P T* – – –	*P P P* – –	*P T T* – –	*P T* – – –	*P T* – – – *	*P* – – – –			
35	*P T* – – –	*P T* – – –	*P T* – – –	*P T* – – –	+ *P T* – –	*P T* – – –	*P T* – – –	*P T* – – –	*P T* – – – *	*P T* – – –	*P T* – – –	*P T* – – –

Dates of exposure: Sept. 5 and 15, 1933.

Serum dilutions: 1:25, 1:50, 1:100, 1:200, 1:400 represented by consecutive −, +, *P* or *T*.

\+ = complete agglutination, *P* = incomplete agglutination, *T* = trace agglutination.

* Indicates calving date.

† Indicates aborting date.

animals to become exposed to infective materials. Of a total of 227 cows studied, 23 remained negative, and 19 never showed more than an incomplete reaction to the agglutination test in a 1:25 dilution. In other words, the results of the agglutination test alone indicated that 41 (18 per cent) of the total were able through some means to resist infection during a long period of time. Certainly the failure of such a large number of animals to become infected when every opportunity was provided, can hardly be accounted for except through the operation of an acquired immunity.

Beach and Humphrey (1935) have contributed the most significant and important data of all on the question of an actively acquired immunity developing in animals after recovery from brucella infection. Thirteen of the animals mentioned in the experiment of Hadley and Welsh (1931), after a period of 5 years during which time they had not again been exposed, were exposed to *B. abortus* by

TABLE 2

Reacting animals reverting to incomplete and negative reactions over a period of 4 to 6 years

Huddleson and Smith (1931)

	AGGLUTINATION TITER			
	+1:25	+1:50	+1:100	+1:200 and 1:500
	Total numbers			
	26	32	24	165
Reverting to partial or trace in 1:25				
Number	8	10	3	9
Per cent	31	31	13	5.5
Reverting to negative				
Number	0	0	2	2
Per cent	0	0	8	1.2

way of the eye and mouth during pregnancy. It may be seen from table 1, in which the results of subsequent serological tests are set forth, that only 1 of the 13 became infected. Of 22 pregnant heifers used as controls and exposed at the same time, 11 became infected and aborted.

The question is often asked, what trend does the agglutination reaction take over a period of years in animals that have been exposed to natural infection? Data obtained by Huddleson and Smith (1931) and set forth in table 2 from a study of animals in a large dairy herd over a period varying from three to eight years serve as a partial answer. Of a total of 189 animals showing an agglutination titer of 1:100 or higher, only 16 (8.5 per cent) reverted to a negative or slight reaction. Of 58 showing a reaction less than 1:100, 18 (31 per cent) either ceased to react or showed only slight reaction in a 1:25 dilution. During the period, 26 of the total never developed a titer higher than 1:25.

When one examines the trend of serum agglutination titers in animals experimentally infected a remarkable difference is found. With three exceptions,

all the animals which Hadley and Welsh (1931) used in the nutritional experiment ceased to react to the agglutination test within two years after the initial exposure. Their data are summarized in table 3.

The identification of adult cattle immune to brucellosis by means other than determining their resistance to artificial or natural infection belongs to the future. There is at present no indication as to what the procedure will be. The measurement of bactericidal antibodies in the serum of animals resistant to the disease does not appear to be a hopeful procedure. Although Irwin and Ferguson (1938) claim to have obtained a reduction in numbers of *B. abortus* in their bactericidal experiments conducted with serum from immune cattle, no one has yet observed complete killing or reduction in virulence of brucella in the presence of blood from such animals.

In recent electrophoretic studies of the mobilities and concentrations of proteins in blood serums from normal and brucellosis-immune cattle, San Clemente

TABLE 3

Comparison of reactor cows on basis of degree of reaction two years after first exposure to B. abortus

Hadley and Welsh (1931)

	HIGH REACTORS (.005 OR HIGHER)		LOW REACTORS (.02 to .005)	
	Number	Per cent	Number	Per cent
Included in statistics	18	100	15	100
Aborted	14	78	4	27
Calved normally	4	22	11	73
Later became non-reactors	14	78	15	100
Became low reactors and remained so	0	0	0	0
Remained high reactors	4	22	0	0
Developed udder infection with *Brucella abortus*	5	28	0	0

and Huddleson (1942) obtained results which would indicate that the factor characterizing the "immune state" is not to be found in the blood serum proteins. No measurable differences were obtained between the serum proteins of immune and normal cattle.

Active immunity in cattle acquired by use of vaccinal agents

As in many other infectious diseases the early attempts toward producing immunity by means of vaccinal agents against bovine brucellosis were conducted empirically and with little knowledge of the natural course of the disease. Little consideration was given to the preparation of the vaccinal agents or the status toward infection of the animals that were injected. It would appear that most of the early attempts at vaccinal immunization of cattle against brucellosis were pointless and a waste of energy.

Those who attempted vaccinal immunization of cattle cannot be criticized too severely for the manner in which the experiments were conducted or for the con-

clusions that were drawn from the data. One must remember that well-controlled experiments on brucellosis in cattle are expensive and that considerable time must elapse before the results are available. In very few instances have investigators been supplied with the required facilities or funds for conducting well-planned and conclusive experiments. They have had to resort to the use of animals not primarily maintained for use in a disease experiment, or to the cooperation of farmers who were willing to "take a chance".

The chief objective in early studies of vaccinal immunization was the prevention of the chief symptom, premature expulsion of the fetus. Little or no consideration was given to the prevention of active infection. This objective was pursued even for many years after Schroeder and Cotton (1941) had demonstrated the presence of brucella in milk from udders of infected cows and its elimination therefrom for long periods of time.

Bang (1906) attempted vaccination of unbred sheep and cattle by means of live and toluol-killed bouillon cultures of *B. abortus*. In the experiments in which the animals received injections subcutaneously before conception, a high degree of protection against the symptom of abortion was noted. Of 15 sheep so treated and exposed by feeding infective material all failed to abort, while four unvaccinated controls aborted. Of 10 goats treated with the toluol-treated cultures and exposed, 7 aborted. One of 4 heifers treated with live cultures and 3 of 5 heifers treated with killed cultures and exposed to infection aborted. Three out of five unvaccinated controls aborted.

In the light of our present knowledge concerning the disease, the results of the vaccination experiments of Bang have little significance. The number of animals used was too small and, furthermore, protection against infection was not considered. Bang's work, however, apparently established a procedure and an index of results in vaccinal immunization which were to be followed for many years in all parts of the world. They are the injection of live cultures before conception, and measuring the results on the basis of the occurrence of abortion.

Stockman and McFadyean over a period of years practiced the injection of unbred animals in England with a massive dose of live *B. abortus* in order to produce protection against abortion. An attempt was also made to observe the immunizing value of killed organisms on animals after breeding. The results obtained in two herds after the use of the two agents are summarized in a report by Stockman (1914) in table 4.

When one attempts to evaluate these data, it must be remembered that the only status of the animals taken into consideration before treatment was whether they had previously aborted or were unbred. No thought was given to determining past or present infection even though the agglutination test was being used for that purpose during this period. In measuring the final results, the criterion for immunity in the treated animals was their failure to abort. The status of the animals as regards infection after treatment was not determined.

Zwick and associates (1920) in Germany made use of various agents in an attempt to prevent abortion in cattle. They employed live and killed organisms alone and each mixed with specific agglutinating serum. Each animal received

the culture from two agar slants suspended in physiological salt solution. It is stated that many of the treated animals had aborted one or more times before treatment. No attempt was made to determine whether either the treated animals and those used as controls were infected before or after the beginning of the study. Their data (table 5) show a considerably lower abortion rate in the animals treated with live cultures than in the controls. In view of the fact that the data were compiled from questionnaires and no apparent attempt was made to determine whether the animals were infected before or after treatment, the results are of doubtful significance.

Jensen (1921) was one of the first to study the efficacy of vaccinating non-pregnant heifers with a live culture to prevent abortion. The heifers were given

TABLE 4

Summary of results of vaccinating cattle against brucellosis in England by Stockman (1914)

METHOD	NUMBER OF ANIMALS OBSERVED	ABORTED	PER CENT
Treatment with live organisms	493	32	6.5
Treatment with killed organisms	110	23	21
Controls	432	101	23.3

TABLE 5

Results of vaccinating cattle against brucellosis in Germany by Zwick and associates (1920)

AGENTS EMPLOYED	NUMBER TREATED	NUMBER ABORTED AFTER TREATMENT	PER CENT
Killed organisms	937	117	12.5
Killed organisms mixed with specific serum	157	20	12.7
Live organisms	482	28	5.8
Live organisms mixed with specific serum	57	3	5
Controls	1,356	245	18.1

from 2 to 3 injections before conception. The study was made on 447 vaccinated and 424 controls in different herds. During the 2 years following vaccination, 23.7 per cent of the treated and 36.8 per cent of the control animals aborted. Jensen was not impressed with the results of his experiment.

Schroeder (1922) in an early attempt to determine the value of vaccines in preventing abortion, injected 11 non-infected, unbred animals subcutaneously with a suspension of live *B. abortus*, supposedly virulent. Four others received repeated injections of killed organisms after conception. There were 8 controls. The animals were bred 60 days after treatment, and then all groups were exposed to infection by feeding infective material from an aborted fetus. Of the 11 treated with live organisms, 1 aborted. Of the 4 receiving killed organisms, 2 aborted. Seven of the 8 controls aborted. While the results of this small experiment indicate that the injection of live organisms before breeding had some

influence in preventing the manifestation of the symptom of the disease, there was no evidence obtained to indicate the absence of infection.

In table 6 are compiled data gathered by Hadley (1921) after injecting a suspension of live brucella organisms into bred and unbred heifers and cows, some of which had previously aborted; the object was to determine the influence of the injection on the incidence of abortion. No mention was made of the status of the animals as regards infections before or after treatment. It may be noted that few controls were used. Even though the number of abortions is slightly greater in the controls than in those treated, it is obvious such data contribute little information on the value of vaccination in producing an active immunity against infection.

Smith and Little (1923), having been impressed with the reported favorable results of live-culture vaccination in non-pregnant animals in England, proceeded to put the theory to test on non-pregnant heifers and one small group of cows. Smith and Little employed whenever possible a newly isolated culture of *B. abortus* as a vaccine, injecting approximately 2 to 5 billion organisms

TABLE 6

Results of vaccinating cattle against brucellosis in Wisconsin by Hadley (1921)

STATUS OF ANIMALS STUDIED	NUMBER OF ANIMALS OBSERVED	NUMBER ABORTED	PER CENT
Unbred heifers	127	28	22
Unbred heifers, controls	24	8	33
Unbred cows	277	28	10
Bred cows	35	6	17
Bred and unbred cows, controls	66	20	30

intravenously or subcutaneously. The experiments were designed more for the purpose of comparing the effects of injecting live organisms into animals with natural exposure to infection. As there is no indication that those treated were again exposed to infection, it must be assumed that when infection occurred it was due to the live organisms injected. Apparently the control animals used in the experiment were exposed to infection from the injected ones, as no mention is made of artificial exposure. All the experiments with one exception, therefore, can hardly be considered as an effort to test the immunizing efficacy of live culture injections. The one exception is a group of 34 heifers treated with a killed vaccine and then exposed to infected animals in one of the control groups. The results obtained from the two methods of exposure are summarized in table 7.

It is interesting to note that the number of abortions in the groups treated with the live culture is considerably smaller than in the control groups. The latter were exposed evidently after they became pregnant. From what is known of the natural course of the disease today, such results are not surprising. There was not, however, a great deal of difference between the percentages that actually

became infected in the two groups. These results are rather surprising and do not agree with the data collected by Edington and Donham (1939).

The results obtained by Smith and Little are sufficiently convincing in demonstrating that the non-occurrence of abortion in inoculated animals is far from being a suitable criterion of freedom from infection, and that the practice of injecting live virulent organisms into non-pregnant heifers or cows with the idea of protecting them against infection is likely to result in more harm than benefit.

Hart and Traum (1925) (see table 7), injected non-infected and non-pregnant heifers and cows subcutaneously with a suspension of live virulent *B. abortus* for the purpose of determining its value as an immunizing agent against in-

TABLE 7

Summary of results of three separate investigations on live and killed culture vaccination of cattle against brucellosis (first pregnancy observations)

INVESTIGATORS	NUMBER OF ANIMALS	TYPE OF ANIMALS (UNBRED)	TYPE OF VACCINE	EXPOSURE	ABORTIONS		INFECTED*	
					Number	Per cent	Number	Per cent
Smith and Little (1923)	64†	Heifers	L§	None	8	13	23	36
	34†	Heifers	K§	To those treated	5	15	9	26
	153‡	Heifers		To those treated	46	30	61	40
Hart and Traum (1925)	20†	Heifers	L	Artificial	0	0	4	20
	10†	Heifers	L	None	0	0	0	0
	16†	Cows	L	None	0	0	10	63
	15‡	Heifers		Artificial	6	40	12	80
Lubbenhusen, *et al.* (1926)	27†	Heifers	L	Natural	2	7	6	22
	33‡	Heifers		Natural	5	15	10	33

* Revealed by examination of fetus or milk.
† Treated.
‡ Controls.
§ L = live virulent, K = killed.

fection and whether the vaccinal strain persisted in the animal body. The heifers received 220 billion and the cows 80 billion organisms. The heifers were exposed to infective materials by the oral route. After injection and breeding, the lactating cows and one group of heifers were not again exposed to infection. Although 4 of the 20 heifers treated were found infected after parturition, none aborted. Of the 15 control heifers exposed by the oral route, 6 aborted and 12 became infected. In a group of 10 heifers treated before breeding and not exposed, no abortions or infections occurred. The 16 lactating cows were injected more for the purpose of obtaining information of the localization of the injected organisms in the udder. Of these, 10 were found later to be eliminating *B. abortus* in the milk, some for a period as long as 6 months.

Lubbenhusen and associates (1926) (see table 7), selected 27 non-infected,

unbred heifers from a large herd of dairy cattle in which brucellosis had been present for several years to test the efficacy of vaccination with live culture in preventing abortion and infection. Thirty-three unbred heifers were selected from the same herd as controls. The heifers were injected with 20 to 60 ml. of a suspension of live organisms from newly isolated cultures made up to a turbidity of 2 on the McFarland scale. The treated and control animals were permitted to associate freely with the naturally infected animals in the herd. During the first period of gestation, 4 abortions occurred in the treated animals, only 2 of which were due to brucella. However, bacteriological examinations made at the time of abortion or parturition revealed 6 (22 per cent) infected. During the first period of gestation, 7 of the 33 controls aborted, 5 of which were due to brucella. Ten (33 per cent) of these became infected.

Lubbenhusen extended the observations on many of the treated and control animals through a second and third period of gestation. At the termination of the experiment covering more than three years, 30 per cent of the treated and 42 per cent of the control animals had shown bacteriological evidence of infection on one or more examinations. Since the treated and control animals had been more or less in association with infected animals from birth, it is quite possible that many of them had developed a considerable degree of active immunity before the experiment was begun. This might easily account for the failure to infect 70 per cent of the treated and 58 per cent of the controls. There is no means of determining whether the treated animals in this experiment became infected from the injection of live organisms or from natural exposure. Since many of the non-pregnant animals used in the infection experiments by Smith and Little (1923) became infected as a result of inoculation it can be assumed that this must have occurred in Lubbenhusen's experiment.

Although the data obtained by Smith and Little, Hart and Traum, and Lubbenhusen demonstrate the futility of trying to obtain active immunity by injecting live virulent organisms into animals without at the same time producing infection, they do, however, reëmphasize the fact that a considerable percentage of non-pregnant heifers and cows possess a high degree of resistance to *B. abortus* infection by any route. Furthermore, for some unexplainable reason the number of abortions that occur after conception in the infected animals is considerably less than the number of actual infections. It would appear from the collected data that if infection is not established by the inoculation of live organisms into animals, most of them subsequently will be found highly resistant to infection. Such results can only mean that it is possible to produce active immunity against brucellosis. But the procedure must be one which will not leave the treated animals infected.

A smooth culture of *B. abortus* was found by the writer (1922) which when injected in large numbers failed to infect guinea pigs or pregnant cattle. This culture, used as a live vaccine (Giltner and associates, 1929), was injected subcutaneously into a large number of non-infected cattle in several herds from 1920 to 1927 to determine if it would cause an increase in resistance against brucellosis. The experiments from 1920 to 1926 were not controlled as no non-infected

controls were employed. The data furnished information only on the harmlessness of the vaccine. Of 293 non-infected animals treated and observed for 14 to 18 months, only 3.7 per cent aborted. In the same herds were 167 untreated animals which were supposedly infected according to the results of the agglutination test. During the same period of observation 25 per cent of these aborted. In 1926 and 1927 many negative control animals were left in the herds in which other non-infected animals were treated. Of the 722 treated, 5.4 per cent aborted during the 14-month period that followed. During the same time 8.1 per cent of 370 controls aborted. There were 644 infected animals in the same herds, but the incidence of abortion in these was also low, it being only 9.9 per cent. Infection had been present for a number of years previously in most of the herds used in 1926–1927. It is known that the number of abortions and new infections decrease in such herds after a period of 3 or 4 years. So it is not surprising to find such a low incidence of abortion in all groups during the year that followed this experiment. The results have no positive significance insofar as protection against infection or abortion are concerned. They do, however, demonstrate that it is possible to inject both pregnant and non-pregnant cattle with a live culture of very low virulence without harmful results. The only serious criticism of this type of vaccine at the time was that it produced specific serum agglutinins which persisted in some instances for more than one year. Their presence in the serum of animals as a result of injecting the vaccine made it difficult to detect actual infection by means of the agglutination test.

The culture used in the previously mentioned studies was dissociated in 1929 to the point where the injection of large numbers into an animal caused no agglutinin response. Then, a series of experiments was begun in cattle in private herds to determine its immunizing value. The experiments extended over a period of 6 years (Meyer and Huddleson, 1936). The results of this study are summarized in table 8. The vaccine was injected into calves, bred and unbred heifers and cows, which were negative to the agglutination test. From 40 to 50 per cent of the negative animals in most of the herds were left for controls.

One may note from the data that the incidence of infection and abortion was unusually low in all groups, that is, treated, controls and infected. Infected animals had been present in most of the herds studied for a number of years previously. In animals from herds of this type, experimental data derived from the employment of agents for immunizing or therapeutic purposes are likely to mislead one into believing that the agent itself was responsible for the favorable results. Fortunes have been made by producers of preventative and therapeutic agents at the expense of cattle breeders by knowingly or unknowingly taking advantage of the natural course of brucellosis in cattle.

When the vaccine made from the dissociated culture was injected into supposedly non-infected animals in herds shortly after the first appearance of the disease, the results which followed were a conclusive demonstration that such a vaccine gave little, if any, protection to animals against the disease.

In 1925 Buck (1930) began a series of experiments designed to determine the efficacy of suspensions of live cultures of *B. abortus* in producing sufficient active

immunity in calves to protect against infection when they become mature. Several cultures of different degrees of virulence were used in the experiments. The final results seemed to indicate that one culture, No. 19, of moderate virulence had possibilities. The 3 calves treated with this culture resisted infection on artificial exposure.

TABLE 8

Summary of results from an attempt to immunize cattle against brucellosis with a live dissociated culture of B. abortus

Meyer and Huddleson (1936)

YEAR	GROUP	NUMBER OF HERDS	NUMBER OF ANIMALS	ANIMALS			
				Aborting		Infected*	
				Number	Per cent	Number	Per cent
1930	Treated		592	13	2.1	70	11.8
	Controls	22	221	17	7.8	55	24.9
	Infected		218	38	17.4		
1931	Treated		611	23	3.7	89	14.6
	Controls	22	159	19	11.9	46	28.8
	Infected		227	50	22.0		
1932	Treated		592	10	1.7	31	5.2
	Controls	27	182	0	0	7	3.8
	Infected		235	36	15.3		
1933	Treated		481	6	1.2	30	6.2
	Controls	27	204	4	1.9	15	7.3
	Infected		213	29	13.6		
1934	Treated		397	12	3.0	34	8.5
	Controls	19	381	4	1.0	26	6.8
	Infected		170	18	10.5		
1937	Treated		504	4	0.7	6	1.1
	Controls	14	251	7	2.7	13	5.1
	Infected		182	29	25.8		

* Based on bacteriological and serological data.

The study begun by Buck was continued by Cotton, Buck and Smith (1934). The results of two experiments are summarized in table 9. In one experiment, 6 heifers near breeding age were treated with strain No. 19. In another, 9 were treated. After breeding they, along with controls, were exposed to infection by way of the conjunctiva. In the first experiment, 1 treated and all 8 controls became infected. In the second experiment, none of the treated, but 7 of 11 controls became infected. Two of the treated animals aborted, but no evidence of brucella infection could be found. The results of these studies led Cotton and his associates to suggest that calves between the ages of four and six months

be treated with strain No. 19 in order to avoid a prolonged serum agglutination titer from the vaccine which would occur if adult animals were injected.

Since 1938 there have appeared numerous reports (see table 9) pertaining to the efficacy of strain No. 19 vaccine in the prevention of brucellosis in cattle in private and experimental herds. Hardenbergh (1939) vaccinated 143 calves, leaving 73 controls. They were maintained in a private herd under natural conditions of exposure. Of the total treated, 3 (2 per cent) became infected after reaching maturity. Four (6 per cent) of the controls became infected. Haring (1938, 1939) Mills (1940), Thomsen (1939), Tompkins (1940), Haring and Traum (1937, 1941) and Winter (1941) have likewise vaccinated a large number of calves that were maintained under natural conditions. All report encouraging results on the efficacy of the vaccine in protecting cattle against infection after they reach breeding age.

TABLE 9

Summary of results from vaccination of calves with BAI strain No. 19 as reported by various investigators

ORIGIN OF STUDY	METHOD OF EXPOSURE	VACCINATED		CONTROLS	
		Total	Number became infected	Total	Number became infected
Cotton, *et al.* (1934)	Artificial	15	1 (7%)	19	15 (78%)
Hardenbergh (1939)	Natural	143	3 (2%)	73	4 (6%)
Mills (1940)	Natural	142	12 (9%)	46	16 (34%)
Thomsen (1939)	Natural	266	9 (3%)	135	34 (26%)
Tompkins (1940)	Natural	24	4 (17%)	32	9 (28%)
Tompkins (1940)	Natural	222*	3 (1%)	0	
Birch, *et al.* (1941)	Natural	35*	10 (29%)	23*	17 (74%)
Mohler, *et al.* (1941)	Natural	8,182†	128 (1%)	0	
Haring and Traum (1941)	Natural	2,872‡	169 (5%)§	1,763	245 (13%)§
Winter (1941)	Natural	968*	63 (6%)	0	0
Rabstein and Welsh (1941)	Natural	642	5 (0%)§	0	0

* First parturition.

† Report covers part of 3 parturitions.

‡ Number of pregnancies.

§ Number of abortions.

Birch, Gilman and Stone (1941) have conducted an exceptionally well-planned and controlled experiment on 35 calves with strain No. 19 to determine its immunizing value and the duration of the immunity. The animals, after breeding, were exposed to infection by placing them in quarters with aborting cows. Of the 35 vaccinated animals and 23 controls, 3 of the former and 14 of the latter became definitely infected during the first pregnancy. Ten of the vaccinated and 17 of the controls also became reactors. Twenty-eight of the vaccinated and 14 of the controls were observed through a second pregnancy. During this period 7 vaccinated and 5 controls became infected. Seven in both groups became reactors.

It is of interest to note that during the first period of this experiment 25 of the vaccinated and 6 of the control animals failed to develop specific agglutinins even though they were in constant association with infected animals. In a personal communication, Birch has informed the writer that 17 out of 18 vaccinated animals and all 6 of the controls that remained negative to the agglutination test through the first pregnancy also kept the same status through the second pregnancy. During a third pregnancy, 5 more of the 18 vaccinated animals became reactors. Of 5 remaining controls all remained negative. None of the animals in question aborted or showed *B. abortus* in the milk. One might raise the question as to whether the animals in the control groups which failed to become infected were naturally immune or had already acquired an active immunity before exposure. Their failure to produce specific serum agglutinins on exposure is similar to what one observes in young calves and to the findings of Beach and Humphrey (1935) in cattle that had recovered from brucellosis. Perhaps the same immunological phenomenon is involved in both natural and actively acquired immunity to brucellosis.

The Bureau of Animal Industry of the U. S. Department of Agriculture has been conducting extensive studies of strain No. 19 in calves in privately owned herds since 1936. Mohler and associates (1941) have summarized the results in the following paragraphs:

"Of the calves vaccinated, 8,182 have now dropped calves involving three pregnancies, of which 5,673 were first, 2,026 were second, and 483 were third pregnancies.

"There were 7,782, or 96.2 per cent, normal parturitions in these herds. Of the latter number 6,526, or 82.9 per cent, calved normally and also were negative on post-parturition test; 399, or 5.1 per cent, calved normally but were positive to the post-parturition test; and 947, or 12 per cent, calved normally and were suspicious to the post-parturition test.

"On the other hand, 310 or 3.8 per cent abortions occurred in these groups, of which 182, or 58.7 per cent, of the aborting animals were negative to the post-parturition test and 99, or 31.9 per cent, were positive to this test, while 29, or 9.3 per cent, of the aborting animals were pronounced suspicious. Consequently, on the basis of the blood agglutination test, only 128, or 1.6 per cent of the abortions occurring in this group of 8,182 animals involved in the three pregnancies could be attributed to brucellosis."

Rabstein and Welsh (1941) have studied the effects of vaccination with strain 19 on animals from the standpoint of the persistence of the agglutinin response as well as the immunizing value. The study included three age groups of which 642 were vaccinated between 3 and 8 months (Group 1), 89 between 9 and 12 (Group 11), and 65 between 13 and 21 months of age (Group III). All of the 796 animals were positive to the serum agglutination test in a dilution of at least 1:200 two weeks following vaccination with the exception of two which remained negative even after being repeatedly vaccinated. Vaccinated calves showing a serum agglutination titer of at least 1:200 were in direct association with susceptible animals and in no instance did the latter show any change in their status.

There was noted a direct relationship between the age of the animal at the time of vaccination and the length of time that a positive blood reaction was retained. Nine months following vaccination, 91 per cent of Group I, 50.5

per cent of Group II, and 20 per cent of Group III were negative to the agglutination test. At 18 months following vaccination, Group I showed 1.4 per cent positive and 3.4 per cent suspicious; Group II contained 6.7 per cent positive and 21.3 per cent suspicious, while Group III had 20 per cent positive and 40 per cent suspicious.

Of the pregnancies recorded on animals vaccinated in this experiment, 172 had one calf, 90 had two, 48 had three, 26 had four, and 8 had five calves each. Out of the total number of pregnancies, ten (1.5 per cent) terminated in abortions of which five appeared to be due to *B. abortus* infection.

From the studies that have thus far been made with strain No. 19 as an immunizing agent against bovine brucellosis, it is reasonable to conclude that, when it is used on calves between the ages of 4 to 8 months, a high degree of active immunity is produced against natural brucellosis infection during the first pregnancy, that active immunity remains even during the second and third pregnancy, that the organism contained in the vaccine used on calves does not

TABLE 10

Summary of a vaccination experiment on cattle conducted by McEwen in England (1937)

YEAR	VACCINATED		CONTROLS	
	Number	Number became infected	Number	Number became infected
1st	109	4 (4%)	98	5 (5%)
2nd	90	2 (2%)	73	14 (19%)
3rd	38	0	29	7 (24%)

establish itself in the animal body to produce a carrier state, that calves and young heifers show an agglutinin titer for only a few months after vaccination.

The proper and continued use of strain No. 19 vaccine should serve a useful purpose in preventing the spread of brucellosis in infected herds and in preventing its occurrence in those herds free from brucellosis. It may play as useful a role in the control of brucellosis (Bang's disease) as the slaughtering of infected cattle.

McEwen (1937 to 1939) in England also has investigated the possibility of using a live culture of *B. abortus* of low virulence for immunizing adult animals as well as those under breeding age. In 1937 appeared the first comprehensive report of his studies in this direction, in which a sufficient number of controls were left to give the results significance. The data from one of McEwen's experiments are presented in table 10. There is little, if any difference between the incidence of infection in the vaccinated and control animals during the first year of observation. McEwen's explanation for this is that the herd employed contained many infected animals which reacted to the agglutination test and possibly many of those that were negative to the test were also infected. If the latter were injected with a vaccinal agent, they would later show evidence of infection and be classified as unprotected by the vaccine.

During the second year that the animals were under observation in McEwen's experiment there was a marked difference between the number that became infected in the treated and in the control groups.

Active immunity in the goat acquired through infection or by use of vaccinal agents

Although the milch goat has been known to be the host and disseminator of *Brucella melitensis* in certain regions since 1905, there are not available any recorded data of either a positive or negative nature pertaining to recovery from the disease and the status of such goats toward a second infection after recovery. The lack of studies in this direction on the goat might appear paradoxical to those not acquainted with the almost insurmountable difficulties which have confronted those who have attempted to study the disease. The type of milch goat inhabiting the enzoötic regions has never been considered of great economic importance, except perhaps to the owner. When to this view is added a poorly organized and inadequately supported animal health service in the same regions, it is not surprising to find that the course of brucellosis in the goat has not yet been explored.

The infected goat continues to be the chief source of brucellosis in human beings throughout the world. But until more information is available concerning the nature of the disease in this host, one cannot expect any considerable reduction in the incidence of the disease in man.

The same situations that have obstructed studies of the infected goat since the Mediterranean Fever Commission was disbanded also have delayed until 1937 any well-directed efforts toward the study of vaccinal agents as a means of producing an active immunity in this animal. In this year Poulding (1939) began a series of experiments in Malta with this object in view. One involved the injection of a suspension of live virulent *B. abortus* into female kids which had been raised from non-infected females. When they had reached the age of $4\frac{1}{2}$ to $5\frac{1}{2}$ months, 12 kids were injected subcutaneously with 2,500 million live organisms. None of the kids become permanently infected from the inoculation as indicated by the disappearance of specific serum agglutinins within 8 months. They were bred on reaching maturity and shortly afterwards, together with 12 controls, exposed to *B. melitensis* infection by permitting them to associate in the same pen with infected female goats that had just aborted. Of the 12 treated goats, one aborted and one failed to conceive. Six of the controls aborted and one failed to conceive. At the time of parturition or abortion, 8 of the treated animals and 8 controls were found infected with *B. melitensis*.

Poulding also studied the immunizing value of another agent on goats. This was a bacteria-free culture filtrate prepared by growing *B. melitensis* in liver broth for a long period of time, then passing the broth through a filter and adding phenol to 0.5 per cent (this agent was reported on previously by Zammitt and Debono (1933)). Two groups of goats were treated with this agent. One group consisted of 15 one-year-old females, and the other of 14 females more than 2 years old the history of which was indefinite. After breeding the two groups they, along with 12 control females, were exposed to infected goats at the time

of abortion. Examinations made on the goats after abortion or after parturition revealed 11 of the 12 controls and 13 of the 29 treated ones infected. Only 2 in the group of older goats became infected.

The results of Poulding's experiments do not indicate that the injection of goats with live *B. abortus* or with a culture filtrate prepared from *B. melitensis* are of any value in actively immunizing them against *B. melitensis* infection. The prevention of the disease in goats is of sufficient importance to warrant continued study in this direction.

Active immunity in the guinea pig and other laboratory animals acquired by use of vaccinal agents

The guinea pig is perhaps the most susceptible small animal of all to experimental infection with any of the three species of *Brucella*. It is known that not more than 25 live *B. abortus* organisms, and 5 or less of *B. suis* when injected subcutaneously, will produce extensive gross changes in the guinea pig tissues within a four-week period of incubation.

In view of its susceptibility, the guinea pig affords an ideal preliminary testing ground for immunizing agents intended for use on large animals and humans. However, if an agent should fail to immunize guinea pigs against infection this does not necessarily imply that it will likewise prove ineffective when used on larger animals. On the other hand, if an agent confers on guinea pigs a high degree of active immunity, there are precedents for expecting the same to obtain in larger animals when it is used in a suitable dose.

For some reason, the guinea pig has been greatly neglected as a test animal for brucella vaccinal agents as indicated by the small number of published reports. Ascoli was the first to attempt the immunization of guinea pigs against *B. abortus* infection by the injection of heat-killed cultures of the same organism. His results were negative. Similar experiments with the same object in view were conducted by Stafseth (1920), Hagan (1922), Schroeder and Cotton (1924) Gwatkin (1933), and Holth (1933). All of these workers also failed to produce an active immunity in the guinea pig against *B. abortus* infection.

McEwen and Roberts (1936) have made the most thorough and comprehensive investigation of all to determine the value of killed and live avirulent culture vaccines for immunizing the guinea pig against *B. abortus* infection. Three types of vaccine were studied, (1) a suspension of live organisms prepared from a culture of *B. abortus* that was relatively non-infectious for guinea pigs, (2) a heat-killed suspension, and (3) a formalin-killed suspension of the same culture. Different groups of guinea pigs were injected with single and multiple doses of vaccine, and were exposed to infection at varying intervals after treatment along with normal controls. The investigators were unable to produce active immunity with the killed vaccines. Their protocols with respect to the immunizing efficacy of the live vaccine contain very conflicting data. In certain experiments, 90 per cent of the treated animals failed to become infected, whereas all controls were infected. In other experiments performed in a similar manner, little, if any difference was found between the incidence of infection in the

treated and controls. McEwen and Roberts considered that loss of the immunogenic antigen in the culture after long cultivation on media might be the contributing factor to the variation in results. An experiment was performed to test this hypothesis by passing the culture through guinea pigs and then comparing its immunizing value with one that had been maintained on culture media. In this experiment 1 of the 10 guinea pigs treated with the animal passage culture became infected on exposure as compared with 4 out of 10 treated with the stock culture. Nine of 10 controls exposed at the same time become infected. The results of this experiment would indicate that loss of immunogenic property of the culture was largely responsible for the variation of results in the previous experiments.

Pennell and Huddleson (1937, 1941) and Stahl, Pennell and Huddleson (1939) have used the guinea pig in determining the immunizing value of such brucella agents as heat-killed organisms, live organisms of an R type, the protein nucleate fraction of the cell, a fraction derived from the cells by treatment with trypsin and tricholoroacetic acid and by trichloroacetic alone. All of these agents failed to protect guinea pigs against experimental infection. A purified and concentrated brucella antiserum was prepared by Huddleson and Pennell (1939) which protected guinea pigs against the toxic effects of the toxic fraction by brucella but failed to protect them against infection. Kolmer, *et al.* (1939) also succeeded in protecting mice against the toxic effects of large doses of brucella organisms through the injection of an antiserum prepared in rabbits, but they report no evidence which would indicate that the mice were protected against infection. Roman (1938) and Renoux (1939) prepared a soluble antigen from *B. melitensis* according to a procedure described by Boivin and Mesrobeanu (1934) and claim to have successfully immunized guinea pigs against *B. melitensis* infection. They found it necessary to inject the soluble antigen along with a live avirulent culture of *B. abortus* in order to obtain a high degree of immunity and, then, only in male guinea pigs. Stahl and Hamann (1941) were unable to confirm the results obtained by Roman or Renoux by preparing a soluble antigen in the same manner and using it in the same dosage in guinea pigs.

Since no one has succeeded in establishing an active immunity against brucella infection in either cattle or guinea pigs by injecting killed organisms, this would indicate that either a mild degree of infection must be produced in the animal before this state is attained, or that the immunizing antigen in the cells is extremely labile and will not withstand the action of physical or chemical agents.

Recently the writer (1942) has succeeded in obtaining from live brucella cells a water-soluble immunizing antigen, and from its study obtained sufficient data to prove the labile antigen hypothesis.

The water-soluble antigen is obtained from live wet cells of either *B. abortus* or *B. suis* by crushing them in a Booth and Green (1938) bacterial crushing mill and removing the crushed cell fragments by centrifugation at high speed. Ethyl ether is added to the supernatant to the point of saturation as a preservative and to kill any live organisms that might not have been removed.

During a period of one and one-half years a total of 247 male and female

guinea pigs, divided into 25 groups, were treated with 0.5, 1 and 2 mg amounts (dry weight basis) of the fraction; each pig receiving the respective dose at intervals of three days. From 15 to 25 days after the last dose all pigs in each group, along with an equal number of controls, were injected subcutaneously with live virulent *B. abortus* in numbers varying from 152 to 3,840. After a period of 4 weeks, the pigs were killed, the internal organs examined for gross evidence of infection, the blood serum tested for specific agglutinins and the tissues cultured for brucella. Of the total number treated only 9 per cent were found infected at the time of necropsy. Of 238 guinea pigs used as controls in the 25 experiments, 151 (63 per cent) were found infected.

The treated guinea pigs not only failed to show the organism in cultures taken from the tissues, but were free from gross tissue changes as well. Specific agglutinins were seldom, if ever found in the blood serum of those free from infection.

Before any degree of immunity could be obtained against experimental *B. suis* infection, using a fraction obtained from *B. suis*, it was necessary to inject guinea pigs with 3 successive 5 mg amounts at intervals of 3 days.

The component in the crushed-cell soluble material that is essential for producing an active immunity is easily destroyed by most antiseptics and by heat. Phenol in a final dilution of 1:200 and merthiolate in a final dilution of 1:10,000 in the material renders it inactive. So also does an exposure to a water-bath temperature of 56°C. for 30 minutes.

The results of this study have furnished proof that an active immunity against brucella infection can be obtained without the intervention of the live organism and that the essential immunizing antigen in the cell possesses labile characteristics.

THE HUMAN BEING

Active immunity acquired through infection

In most of the studies of human brucellosis more emphasis has been placed on the clinical cases, their detection, and attempts at treatment rather than on a study of those that fail to show clinical manifestations of the disease even though the results of various diagnostic tests furnish positive proof that infection once existed. The golden opportunity to accumulate facts relating to the nature and occurrence of acquired immunity, that was missed from failure to study cases after recovery from infection whenever they occurred in animals, has again been presented and lost when similar cases and groups were found in human beings.

Proof of an acquired immunity to brucellosis developing in human beings after a clinical or sub-clinical manifestation of the disease is based largely on observations of specific serum and allergic tests and the interpretation given to the findings. The available data are of sufficient extent to be highly significant if they are interpreted by deductive analysis in terms of acquired immunity.

The laboratory diagnostic procedures which have been most widely used and which furnish the most reliable information toward confirming a diagnosis of

human brucellosis are the blood culture, the serum agglutination, the whole blood opsonic, and the skin sensitivity tests. The results of the last three also have been employed in obtaining valuable information relative to previous clinical and sub-clinical infection. The opsonic test, when properly performed is a valuable aid to the interpretation of a positive agglutination or skin test. It was pointed out by Huddleson, Johnson and Hamann (1933) that when most of the neutrophiles in citrated whole blood of a normal individual show a marked ingestion capacity for brucella cells, this finding usually signifies a previous infection and a high degree of resistance on the part of the individual in question to subsequent infection. That there are exceptions to this interpretation is now well known. That is to say, the blood leucocytes of a small percentage of clinical cases will also show a high degree of phagocytosis and likewise the leucocytes of a small percentage of those who have long since recovered will show a low ingestion power.

It may be the contention of some that the presence of demonstrable brucella antibodies in the blood serum or the presence of specific skin sensitivity to brucella allergens in a healthy individual, with or without a history of clinical manifestations of the disease, constitutes evidence of continued infection. There is, of course, a logical basis for their reasoning, which is that both acute and ambulant cases show the same detectable serum antibodies or skin sensitivity reactions. But when the same type of reactions can be induced in human beings by the injection of the proper agents without producing the disease itself, is it not logical to believe that after the live organism enters the body it could also induce specific serum antibodies and skin sensitivity and then be destroyed with signs of little, if any, clinical manifestation of infection? The writer does not wish to imply that the commonly known brucella serum antibodies and skin sensitivity, when found in a normal individual, necessarily play an important role in active immunity. At the present time they can only be interpreted as some of the signs which constitute proof that the body tissues of an individual have reacted to the live organism, and, when present after recovery from the disease or in the absence of any known history of the disease, indicate active immunity.

Considerable data have been accumulating for a number of years from the use of the specific tests on large groups of human beings. The results when carefully analyzed point in the direction of their significance. There is also circumstantial evidence of an epidemiological nature that can only be interpreted as proof that immunity to brucellosis is acquired by many individuals from exposure to the organism.

Let us first examine the circumstantial evidence. In doing so, it is realized that there are many who will view such evidence with skepticism, as unworthy of scientific consideration. Nevertheless, many important precedents can be cited in the history of medicine for not ignoring in this instance the circumstantial evidence pointing to the existence of an acquired immunity to brucellosis.

Since the development of accurate diagnosis procedures, from 200 to 500 cases of brucellosis have been diagnosed in the inhabitants of Malta each year; the

yearly population averaging approximately 220,000 over the past 50 years. Since it is known that the disease on the Island is acquired from drinking raw goat's milk or eating products from such milk, that approximately 15 per cent of the goats are infected with *B. melitensis* and that, up to 1938, 90 per cent of the people consumed the milk in the raw state, it must be admitted that the number who are exposed each year, and year after year without showing clinical manifestations, is far greater than the number who do. This extreme difference in the number exposed and in the number known to become clinically infected, instead of being considered in its proper light, has been used by prominent individuals, not only in Malta but in other countries as well, as an argument against infective milk being one of the chief means of conveying brucellosis to human beings.

Since *B. abortus* and *B. suis* and their respective hosts, the cow and the hog, have become implicated in the occurrence of human brucellosis in the United States and other countries, the same situation as regards wide differences in the number exposed to those clinically infected has been apparent. And as in Malta, individuals have used this widely acknowledged situation to bolster the thesis that the consumption of animal products containing either *B. abortus* or *B. suis* is not the chief source of human brucellosis. Fortunately, there exist data from several surveys and studies on the human being with respect to *B. abortus* and *B. suis* which, when added together and analyzed, supply evidence that more infections have occurred than are apparent. Most of them were undoubtedly of a sub-clinical nature and would not have been detected without the use of specific tests applied to the blood serum or in the skin. That these individuals acquired a substantial immunity to the disease after the initial infection is borne out by their subsequent histories.

If one undertook the task of obtaining information that would aid in explaining why many individuals fail to develop the disease even though they are exposed repeatedly to infective materials, he would first examine those in occupations or professions who come in contact more often than others with brucellosis-infected animals or materials. The results of such examinations are well illustrated in the five following investigations.

Huddleson and Johnson (1930) were among the first to collect enlightening data on such a group, which served as the initial answer to the question of the occurrence of acquired immunity to brucellosis in human beings. They examined the blood of 49 practicing veterinarians for brucella agglutinins and questioned the individuals as to any past history of clinical manifestations of the disease. Of the total number examined, 28 (57 per cent) showed serum agglutinins in a titer of 1:50 or above. Only 3 of these gave a past history of a symptom-complex characteristic of the disease. From many of the veterinarians who showed serum agglutinins, it was learned that small eruptions appeared on the skin of the arm used in removing retained placenta from cows that had aborted. At the same time there also occurred a general malaise not unlike the symptoms of the disease. That these reactions were manifestations of a brucella allergy was proved by the injection of a specific allergen into the skin of the same

individuals and producing the same reactions. The presence of serum antibodies and skin sensitivity in such a high percentage of the individuals examined furnished indirect proof that they were at one time infected with brucella. Their failure to show clinical evidence of the disease, even though exposed repeatedly, can only mean that the initial infection was of a slight nature and caused the development of a high degree of acquired immunity.

Additional data, which showed that a large percentage of veterinarians develop signs of sub-clinical infection on exposure to infective materials and subsequently are never subjects of the disease, were obtained by Thomsen (1931) in Denmark. He found that 94 per cent of all veterinarians who had been in cattle practice for more than one year, showed specific antibodies for brucella in the blood serum, and a high percentage in practice for several years also show brucella skin allergy after removing retained placentas from infected cows. Thomsen believed that

TABLE 11

Comparison of the results of the agglutination test with results of other laboratory tests on 41 clinical and 49 subclinical cases of brucellosis

Huddleson and Munger (1940)

MAXIMUM AGGLUTINATION	41 CLINICAL CASES				49 SUB-CLINICAL CASES			
	Neg.	1:25	1:50	1:100 or higher	Neg.	1:25	1:50	1:100 or higher
Titers (no.)	4	12	7	18	26	8	6	9
Blood culture, positive	3	12	7	13	1	0	1	1
Brucellergen test, negative	0	0	0	0	1	1	1	0
Brucellergen test, positive	4	12	7	18	25	7	5	9
Phagocytosis, low or moderate	3	10	6	14	16	6	4	7
Phagocytosis, marked	1	2	1	4	0	1	1	2
Phagocytosis, negative	0	0	0	0	10	1	1	0

the results of the test in the absence of the disease indicated the existence of an acquired immunity which came from exposure to infective material after entering practice.

Huddleson and Munger (1940) examined a total of 349 college students during a small epidemic of brucellosis due to *B. melitensis* for possible evidence of exposure to infection. Four tests were employed, namely, the agglutination, opsonic, skin sensitivity, and blood culture to determine the occurrence of clinical or sub-clinical infection. Not all those examined had had the same opportunity to become exposed. The number, therefore, that showed a positive reaction to one or more of the tests cannot be statistically analyzed in terms of the entire group. Of those examined, 41 were definite clinical cases. There were also 49 others in whom one or more of the tests indicated infection, but who continued to remain symptom-free. These data (see table 11) indicate that when a group of individuals is equally exposed to brucella infective material, there will be found among them as many who show evidence of infection without clinical manifestations as those who do.

Dooley (1932) was prompted to examine the blood serums of 263 boys in a boys' school for brucella agglutinins after two of them developed clinical brucellosis due to *B. abortus*. Of the total number examined, 41 per cent were found to show agglutinins in titers ranging from 1:40 to 1:12,000. All the boys, with two exceptions, remained in good health during a one-year observation period.

Wilson (1932) examined two groups of veterinarians, one of which had had ample opportunity to be exposed to *B. abortus* by contact with infected animals, while the other group had no history of such contacts. The agglutination test

TABLE 12

Results of specific brucella tests on groups of humans obtained by various investigators

OBSERVER	GROUP OBSERVED	LOCATION	TEST EMPLOYED					
			Agglutination		Opsonic		Skin allergy	
			Number tested	Per cent positive	Number tested	Per cent positive	Number tested	Per cent positive
Meyer, *et al.* (1934).....	V*	California			100	74	58	60
Huddleson, *et al.* (1937)...	V	Michigan	49	57	20	95	50	20
Lerche and Roth (1933)...	V	Germany					44	93
Dubois and Sollier (1931).	V	France					14	29
Jordan (1931).....	V	Iowa	120	45				
Kitselman (1932).....	V	Kansas	88	30				
Meyer, *et al.* (1934).....	P*	California			161	67	615	50.6
Huddleson, *et al.* (1933)...	P	Michigan	167	11	167	24		
Jordan (1931).....	P	Iowa	220	31				
Heathman (1934).....	P	Minnesota	1,096	6.9			1,096	55.0
Lentze (1930).....	F*	Germany	57	23				
Makkawejsky, *et al.* (1931).....	F	Russia	354	7.6				
Meyer, *et al.* (1934).....	F	California			30	57		
Meyer, *et al.* (1934).....	C*	California			103	35	54	11
Levin (1930).....	C	Oregon					269	3
Huddleson, *et al.* (1933)...	C	Michigan					240	12.5
Huddleson, *et al.* (1937)...	C	Michigan	8,124	1.2	8,124	8.0	8,124	10.3
Keller, *et al.* (1938).....	C	Tennessee	1,247	2.1			1,247	4.8
Angle, *et al.* (1938).....	S	Missouri					7,122	8.7

* V = veterinarians, P = packing house employees, F = farmers, C = clinic and hospital patients, S = school children.

made on the blood serums of 63 of the former revealed 24 per cent showing a positive reaction, while in the latter group of 35 only 3 per cent showed specific serum agglutinins. Little, if any, positive evidence was obtained that those who reacted to the agglutination test had at one time been clinically infected with brucella.

Since 1930 there have been many investigations of a survey nature conducted on many groups of people by use of specific serum and allergy detecting tests chiefly to learn more about their significance and usefulness in detecting those who were once infected with brucella. The results that have been obtained in

a few of the surveys made on groups of human beings through the application of one or more of the specific tests are summarized in table 12. The groups are representative of those whose occupations bring them in close contact with hosts of brucella and those in the general population. The results of the tests clearly demonstrate that the greater the opportunity for exposure to infection, the larger will be the number of individuals who will show evidence of having been infected. The small percentage of positive reactions found by the agglutination test in comparison to the higher percentage obtained by the skin test is highly significant evidence that the majority had passed through the infection stage long before the tests were made. From the studies that have been made on many groups of people it has been shown that only a few of those, who have long since recovered from infection and who continue to show skin sensitivity, will show serum agglutinins in a titer of 1:20 or above. And as in the case of large animals, such individuals seldom, if ever show clinical evidence of infection or a considerable increase in serum agglutinins on repeated exposure to natural infection.

The proof that groups such as veterinarians, certain packing-house employees, farmers and many laboratory workers, who are free from clinical evidence of infection, but react to one or more of the brucella tests, are actively immune to the disease rests largely on the evidence that has been adduced from their histories. It is well known that an individual, who shows by the negative character of all tests no indication of ever having been infected, cannot long work with infected animals or infective materials without showing some evidence of either clinical or sub-clinical infection. So, when groups of people such as veterinarians continue to work year after year in the midst of infective materials without developing the disease, it is clear evidence that the initial infection, after its termination, confers upon the individual a high degree of immunity. It is true that such persons may show an allergic reaction, the symptoms of which closely resemble the disease, on exposure to the organisms or the protein of the organism by way of the skin, digestive or olfactory tract. The allergic reactions are seen more often in veterinarians and certain laboratory workers than in other groups and have been time and again mistaken for symptoms of the disease.

When one carefully analyzes all the data that have been collected in the numerous surveys on a large number of people by use of the specific tests in terms of their histories, the evidence in favor of the occurrence of an acquired immunity to brucellosis after clinical or sub-clinical infection is overwhelming. The only tests thus far employed that have the semblance of indicators of the immune state are the skin sensitivity and opsonic tests. Since the infected also show skin sensitivity and opsonins it is obvious that these tests alone cannot always be relied upon as critera of active immunity. The real indicator of active immunity to brucellosis in the case of the human still awaits discovery.

Active immunity in man acquired by use of vaccinal agents

The many attempts that have been made at vaccinal immunization of human beings against brucellosis are good examples of wasted effort and stabs in the dark. The attempts have centered around the use of suspensions of one or

more of the species of *Brucella*, killed by heat or chemical agents. Instead of employing suitable controls in order to gauge the effectiveness of the vaccine, the investigators drew a comparison between the incidence of the disease in similar occupational groups before and after treatment. In view of what is known about the occurrence of sub-clinical or latent infections, it does not seem possible that anyone would attempt to evaluate the efficacy of a vaccinal agent from data obtained in this manner.

One of the most recent studies of a heat-killed vaccine involving a large number of people is that of Dubois and Sollier (1938). They treated with a heat-killed vaccine 4,022 persons whose occupations exposed them to infected animals or infective materials from such animals. Only two cases of infection occurred in the entire group during a period of observation varying from 1 to 8 years after treatment. Meyer and associates (1934), on the other hand, have presented the history of one individual who failed to receive any protection whatsoever from three injections of a heat-killed vaccine. They state emphatically that there is no experimental proof that vaccines prepared from killed bacteria are capable of conferring the least degree of protection against brucellosis. It may, therefore, be said that there is as yet no safe and effective vaccine for human use. A suitable one would occupy an important place in the prevention of the disease in those in hazardous occupations and in the general population of countries where sanitary practices meet with almost insurmountable obstacles.

RECAPITULATION

A critical analysis of the data now available pertaining to immunity in brucellosis furnishes convincing proof that such a state can be demonstrated in animals and man. In the bovine there appear to be two types of immunity, (1) a so-called "natural immunity" that functions in young animals up to or near the beginning of ovulation, (2) an acquired immunity usually developing in unbred heifers and cows and requiring for its stimulus the presence of live organisms in the body or the injection of a labile immunogenic constituent of the bacterial cell.

When virulent organisms enter the body of young animals under natural conditions or are injected in small numbers, they remain for only a short time and cause little, if any, reaction on the part of the tissues. Only when large numbers of organisms are injected is sufficient reaction provoked to cause the appearance of serum antibodies in a high titer, and even then such antibodies persist for only a short time.

A large percentage of normal non-pregnant heifers and cows, when exposed to brucella infective material instead of becoming actively infected, develop a high degree of active immunity against subsequent exposures. From the small amount of data now available it would appear that the immunity acquired through the infective process persists for more than 5 years.

When human beings are exposed to brucella, a large percentage of those exposed fail to show any clinical evidence of the disease, but do develop specific serum antibodies and skin sensitivity, and in some instances to the same degree as those clinically infected.

When antibodies or skin sensitivity are found present in healthy persons a

state of active immunity is indicated. No convincing data have yet appeared pertaining to the presence of active immunity in persons who fail to show a reaction to one or more of the specific tests.

Although the demonstration of specific antibodies in the blood serum or specific skin sensitivity in normal human beings cannot be considered as the final and always certain means of detecting those who are actively immune to brucellosis, they will have to be accepted and utilized for what they are worth until the real indicator of active immunity can be discovered.

In the light of our present knowledge it appears that the commonly known brucella serum antibodies are by-products arising from the reaction between antigen and body tissues, and have no proved function in the active immunity mechanism.

From the facts now available it may be stated categorically that the possibility of animals and human beings acquiring an active immunity to brucellosis through infection is no longer a hypothesis but a demonstrated fact.

There is now conclusive proof that an active immunity can be established in adult cattle against brucellosis by injecting them as calves with live *B. abortus*, the vaccine being prepared from a culture of low virulence. The duration of the immunity produced in this manner has not been determined conclusively.

Although it seems highly probable that human beings could be immunized against brucellosis, there is up to the present time no convincing proof that an agent for this purpose has been developed.

The possibility of an active immunity occurring in swine to *B. suis*, the infecting species, has not been considered in this review due to the fact that no information is available. Very little is known even about the course of the disease under natural conditions. It has long been recognized that swine brucellosis is an important economic disease, and that the infected hog is a reservoir from which the disease is acquired by human beings and other animals. The well-planned research program on swine brucellosis now being undertaken at federal and state experiment stations, if continued, should furnish information on the course of the disease and its immunological aspects that is now so badly needed.

Since there are three known species of *Brucella*, and each may infect species of animals other than the one in which each is commonly found, it would seem essential to the successful application of measures to control the disease to know whether recovery from an attack of one species of *Brucella*, or vaccinal immunization with one species will afford protection against the other species. In the case of human beings, it has been observed repeatedly in many laboratories that those who have developed an immunity as a result of a clinical or sub-clinical infection with one species, may freely work with the others without becoming infected. This observation has been made so many times in several laboratories that it now seems certain that an immunity which is produced by one species will protect against an infection by the other two. There are also experimental data which show that the guinea pig can be immunized against *B. abortus* infection by treatment with the crushed cell fraction derived from *B. suis* as well as from *B. abortus*.

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